CONTRIBUTION TO THE EMBRYOLOGY OF MUILLA, WITH A REMARK ON THE TAXONOMIC POSITION OF THE GENUS

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This paper is the first of an intended series on the tribe Allieae, subfamily Allioideae, of the family Liliaceae (*sensu* Krause, 1930) or Amaryllidaceae (*sensu* Hutchinson, 1959). This series will be a continuation of previous studies within the Liliaceae (Berg, 1958; 1959; 1960; 1962a; 1962b).

Muilla (anagram of *Allium)* is a small genus of five species native to the southwestern United States and Mexico (Ingram, 1953). Only one species, *M. maritima* (Torr.) Wats., was studied. Megasporogenesis, embryo sac development, and early endosperm development is described, as well as the accompanying changes in the ovule. The taxonomic significance of this new information is considered and inconsistencies with the present taxonomic position of *Muilla* are pointed out.

No information about the embryology of *Muilla* is available in the literature.

Material was collected from plants growing in an alkaline flat by the Pole Line Road, 1.1 miles north of Davis city limit, Yolo Co., California (DAV). Ovaries were fixed in Belling's modified Navashin fluid and dehydrated with tertiary butyl alcohol. Sections were cut at 10 and 12 microns and stained with safranin and fast green.

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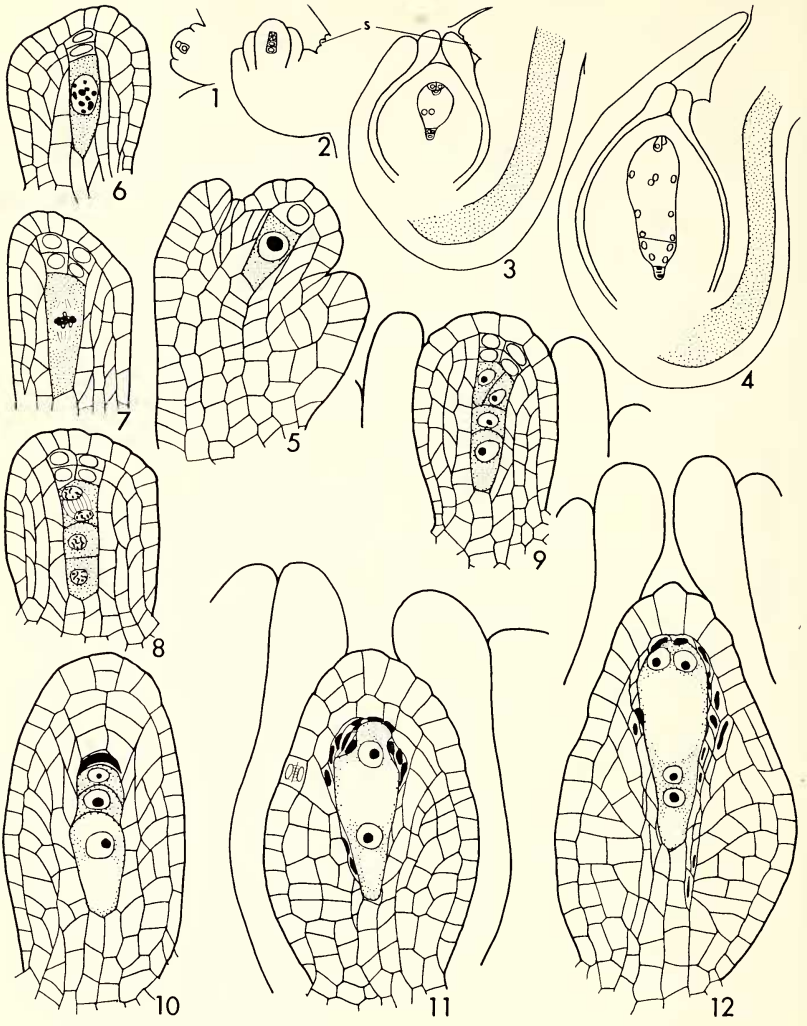
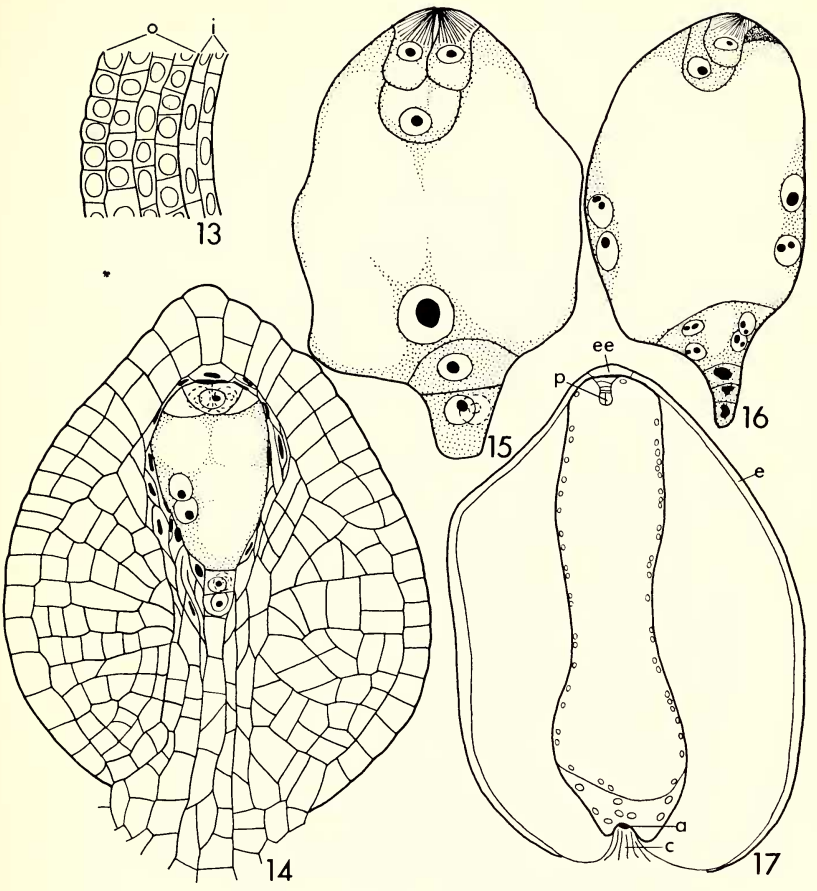
Observations

Ovule. The ovule is anatropous, bitegmic, and crassinucellate (fig 3). At the early megaspore mother cell stage, it is short and nearly straight (fig. 1); at the megaspore tetrad stage it has become curved back approximately 90° (fig. 2); the anatropous stage is attained at ca. the four-nucleate embryo sac stage. There is no distinct funiculus. Raphides were not observed in the ovule, but may develop in the seed, since they are common in the ovary wall.

The inner integument develops near the apex at approximately the stage when the archesporial cell divides (fig. 1). Throughout development to the proembryo stage, the inner integument is two cells thick, except at the apex where it becomes three to five cells thick. The cells at the apex are more or less isodiametric whereas the remainder are elongate, being two to three times as long as broad at the time of fertilization (fig. 13). The outer integument begins to develop at the young megaspore mother cell stage and by the time of meiosis is well defined. It is three cells thick early in its development but soon develops into a structure that is four to five cells thick, the cells being approximately



FIGS. 1–12. 1, Ovule from a bud which was still underground, showing megaspore mother cell, primary parietal cell, and young annular inner integument; 2, Ovule from a bud located at the soil surface, showing a T-shaped tetrad and short integuments; 3, Ovule from a young bud, its pedicel still short, showing embryo sac before fusion of polar nuclei with inner integument closed above nucellus; 4, Young seed, showing helobial endosperm of four plus eight nuclei (all nuclei projected into one plane). Note characteristic elongation of outer integument; 5, young ovule, showing the arrangement of nucellar cells and an early stage of integument development; 6, Nucellus at a somewhat later stage with two parietal cells and megaspore mother cell in diakinesis; 7, Nucellus with two tiers of parietal cells and megaspore mother cell nucleus in metaphase I; 8, Linear tetrad in which division in upper dyad



FIGS. 13–17. 13, Integuments shortly before anthesis. *i:* inner integument, *o:* outer integument; 14, Nucellus from a medium-sized bud, showing numerous periclinal walls in upper part of nucellar epidermis, characteristic radial rows of cells in lower part, and a central core of narrow cells directly below the embryo sac; 15, Mature embryo sac as seen at anthesis; 16, Young helobial endosperm, both chambers four-nucleate. One synergid destroyed and antipodals degenerating; 17, Endosperm of 128 plus 8 nuclei surrounded by greatly enlarged nucellus. *a:* remnants of antipodal cells, *c:* central core of nucellar cells, *e:* one-layered nucellar epidermis, *ee:* two-layered nucellar epidermis, *p:* proembryo. Figs 13–15 ×360, fig. 16 ×90, fig. 17 ×56.

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cell is slightly delayed; 9, T-shaped tetrad; 10, Nucellus showing first periclinal wall in epidermis, three tiers of parietal cells above the megaspores, and germination of lower megaspore; 11, Two-nucleate embryo sac stage; 12, Four-nucleate embryo sac stage. All parietal cells are absorbed. Note characteristic radial arrangement of cells in nucellus, *s:* stigmatoid tissue. Figs. 1–3 ×90, fig. 4 ×56, figs. 5–12 ×360.

isodiametric at the time of fertilization (fig. 13). The inner integument closes above the nucellus approximately when the embryo sac has reached the four-nucleate stage (figs. 11–12). The outer integument remains short for a long time so that the micropyle is formed by the inner integument only (fig. 3). However, after fertilization the outer integument grows considerably to form an unusual elongation towards the placenta (fig. 4).

The nucellus is medium-sized and completely surrounds the embryo sac at the time of fertilization (fig. 3). By this time the nucellus is approximately twice as long as the embryo sac and nearly as broad as long. The nucellar epidermis undergoes periclinal divisions, the first of which are found shortly after the germination of the functioning megaspore (fig. 10). These periclinal divisions in the nucellar epidermis continue until the time of fertilization but are rstricted to the micropylar half of the nucellus only (figs. 11–12, 14), where a multiple epidermis of two, rarely three, layers eventually forms. Most of the body of the nucellus develops from the cells immediately beneath the epidermis. As the ovule grows, these subepidermal nucellar cells divide repeatedly, both periclinally and anticlinally, to form a characteristic pattern of radiating rows of cells (figs. 11–12, 14). After fertilization, more cells are added in this region and, as the ovule grows, most nucellar cells enlarge greatly. However, those of the central core region at the chalazal end of the embryo sac and those of the multiple epidermis at the micropylar end of the embryo sac remain small, and contribute only insignificantly towards the size of the post-fertilization nucellus (fig. 17).

Archesporium and Megasporogenesis. Only one archesporial cell is present in the ovule of *Muilla maritima*. Before the bud appears above ground in spring, the archesporial cell has divided to form the megaspore mother cell and a primary parietal cell (fig. 5). The primary parietal cell divides anticlinally, or periclinally, while the ovule is still in the megaspore mother cell stage (fig. 6). The two cells resulting from this division divide further to give rise to several parietal cells arranged in three, sometimes four, tiers above the megaspores (figs. 7–10). Some of the parietal cells remain until the two-nucleate stage in embryo sac development (fig. 11), after which all are absorbed by the developing embryo sac (fig. 12).

Megasporogenesis begins with the elongation of the megaspore mother cell (figs. 5–6). The spindle of the first meiotic division is longitudinally oriented and located a little above the middle of the cell (fig. 7). Nuclear division is followed by wall formation resulting in a dyad, the micropylar cell of which is slightly smaller than the chalazal one. Each dyad cell divides, the chalazal one normally by a cross-wall, the micropylar one by a wall which is sometimes horizontal but more often longitudinal or oblique (figs. 8–9). The division in the upper dyad cell may be slightly delayed (fig. 8). The tetrad of megaspores will be linear or T-shaped. At the time of formation, the two lowermost megaspores are

about equal in size and a little larger than the uppermost two (fig. 8). Embryo sac development is monosporic. The upper three megaspores degenerate, normally starting with the micropylar one (fig. 10) and proceeding progressively downward. In all ovules observed, with one possible exception, the chalazal megaspore functioned.

Megagametogenesis and Megagametophyte. By enlargement and vacuolization, the functioning megaspore becomes the embry sac mother cell (fig. 10). Normally two vacuoles are formed, one above and one below the nucleus. As the embryo sac grows, the upper vacuole enlarges to become the central vacuole (figs. 11–12) while the lower one remains small and finally disappears (figs. 11–12, 14–15). The eight-nucleate stage is reached by three successive divisions of the embryo sac mother cell nucleus (figs. 10–12, 14). The micropylar part of the embryo sac increases more in size than the chalazal part (figs. 11–12), the latter eventually forming a small, narrow portion where the antipodals lie (figs. 14–15).

The mature embryo sac (fig. 15) is approximately one-and-a-half times as long as broad and of typically ovoid shape. The synergids are similar in size and appearance and are smaller than the egg. They are pear-shaped, each with a vacuole in the chalazal end, a nucleus located immediately above the vacuole, and a filiform apparatus radiating out from the point of attachment. The large egg cell has the nucleus in its lower end and is highly vacuolized. The three antipodal cells are arranged differently in different embryo sacs; most often two are found at the same level with the third one above (fig. 15) or below (fig. 14). Occasionally they are all in a row (fig. 16). The antipodal cells are as large as, or somewhat larger than, the synergids. They begin to degenerate a little before or during fertilization, but they are still obvious in early endosperm stages (figs. 16–17). The polar nuclei fuse before fertilization to form a large secondary nucleus, which lies close to the antipodals (figs. 14–15).

Fertilization and Endosperm Development. After pollination the pollen tube grows down the open stylar canal, which is lined with papillate stigmatoid tissue. The stigmatoid tissue of the style connects with a similar tissue on the placentae, some of which is apparent in figs. 2 and 3. One of the synergids is destroyed when the pollen tube enters the embryo sac. The other remains until more than a hundred nuclei have formed in the endosperm. Double fertilization occurs. Fusion between the secondary nucleus and one of the male nuclei takes place before the fusion in the egg nucleus. Apomictic phenomena were not observed.

The primary endosperm nucleus starts to divide before the zygote. Endosperm is helobial. The first nuclear division takes place in the chalazal end of the endosperm cell. It is followed by the formation of a transverse wall, which cuts off a smaller chalazal chamber from the larger micropylar one. In both chambers free nuclear divisions occur, and in the bginning they are synchronous. The following stages were

observed (nuclei of micropylar chamber/nuclei of chalazal chamber): 1/1, 2/2, 4/4, 8/4, 16/4, 32/4, 8/8, 16/8, 128/8. To judge from this series, nuclear divisions occur simultaneously in the two chambers until four nuclei are present in each (fig. 16). Then development slows down in the chalazal chamber: after eight, or possibly sometimes only four, nuclei have been formed in this chamber, nuclear divisions apparently cease. The nuclei already present become hypertrophied to some extent and the chamber shows signs of degeneration. Since later stages were not present in our material, the ultimate fate of the chalazal endosperm chamber could not be ascertained. In the micropylar chamber, nuclear divisions continue (figs. 4, 17), and the endosperm proper is wholly or largely developed from this chamber.

Discussion

A full discussion of embryologic similarities and dissimilarities between the genera of the tribe Allieae must wait until data, comparable to those presented above for *Muilla,* are available for the remaining genera of the tribe. Such a discussion will constitute the final paper of this series. However, in a preliminary way, a few interesting facts can be pointed out.

The tribe Allieae, as conceived by Krause (1930), consists of 16 genera, some of which later have been united. Embryologic information has, so far, been produced only for *Gagea, Allium, Nothoscordum, Brodiaea,* and *Leucocoryne. Gagea,* furthermore, is now generally considered to be a member of the subfamily Lilioideae (Berg, 1962b). The information on *Brodiaea* (which is at present under investigation by the senior author) is limited to a record of helobial endosperm in *B. peduncularis* (Stenar, 1949). We are left, therefore, with only three genera for our preliminary survey, viz. *Allium, Nothoscordum,* and *Leucocoryne* (table 1).

Of special interest is the lack of a parietal cell in all these three genera, as opposed to the presence of a multicellular parietal tissue in *Muilla.* Stenar (1932, p. 39) points to the absence of a parietal cell as a typical feature of the Allieae. The bisporic type of embryo sac development in *Allium* and *Leucocoryne* and the nuclear type of endosperm in *Allium* constitute additional important deviations from the *Muilla* pattern. *Allium* differs from *Muilla* also in the shape of the embryo sac, in the number of cell layers in the integuments, in the position of the secondary nucleus, and in other minor features. *Nothoscordum* is most similar to *Muilla,* but this genus is also set somewhat apart from *Muilla* by the nature of its nucellus and the nature of its synergids, as well as by the absence of a parietal cell, and the occurrence of an *Allium* type embryo sac in one of its species.

The "key combination" (Berg, 1962b) of "+ N He" (parietal cell present, normal- or *Polygonum* type embryo sac, and helobial endosperm) that characterizes *Muilla* is not found in any other genus of the

TABLE 1. EMBRYOLOGICAL COMPARISON OF SOME GENERA OF THE TRIBE ALLIEAE

(+ = yes or present, 0 = no or absent)

	Allium	*Nothoscordum*	*Leucocoryne*	*Muilla*
No. of cell layers in inner integument	3–4		2	2
No. of cell layers in outer integument	4–10		2–4	3–5
Periclinal walls in nucellar epidermis	+	+	+[6]	+
Numerous such walls	0	0	0[6]	+
Bulk of nucellus produced from nucellar epidermis	0	0	0[6]	0
Nucellar apex penetrated by embryo sac	0[1]	0	0[6]	0
Parietal cell	0	0	0	+
Type of embryo sac development	Allium[2]	Polygonum[3]	Allium	Polygonum
Lower spore functions	+	+	+	+
Length/width of embryo sac	4/2	3/2	3/2	3/2
Synergid filiform apparatus	0&+	+		+
Synergids vacuolated	0&+	0		+
Polar nuclei fuse prior to fertilization	0&+	+[5]	+	+
Endosperm type	Nu	He[4]		He
References	Håkansson, 1951 Jones and Emsweller, 1936 Messeri, 1931 Murphy, 1946 Rao, 1940 Schnarf, 1931 Schürhoff, 1922 Weber, 1929	Eckles, 1941 Håkansson, 1953 Messeri, 1931 Schnarf, 1931 Stenar, 1932	Cave, 1939	

[1] Weber (1929), Håkansson (1951), and Hasitschka-Jenschke (1958) state that the nucellus is partly destroyed when the embryo sac is at the four-nucleate stage.

[2] Weber (1929) reported both mono- and tetrasporic embryo sac development for *Allium carinautm* and monosporic embryo sac development for *A. paradoxum* However, the significance of these variants is questinable because both of these species reproduce by bulblets and either the embryo sac does not develop or, if it does, the embryo does not survive. All normally reproducing, amphimictic species show *Allium* type embryo sac development.

[3] One of the three species of *Nothoscordum* included in this table, *N. fragrans,* is different from the others in that the embryo sac development is of the *Allium* type (Messeri, 1931; Stenar, 1932). However, it is of interest to note that this species shows adventive polyembryony and may present a similar case as *Allium carinatum* and *A. paradoxum,* i.e. an abnormal deviation within its genus.

[4] Schnarf (1931, p. 242) states that nuclear endosperm has been encountered in *Gagea, Allium,* and *Nothoscordum,* a statement that as far as *Nothoscordum* is concerned is contrary to Stenar's (1932) observations.

[5] Polar nuclei fuse after fertilization, according to Håkansson (1953).

[6] Marion Cave, personal communication.

tribe Allieae. On the other hand, *Muilla's* key combination occurs in several genera of four other subfamilies of the Liliaceae *sensu* Krause, namely the Melanthioideae (Schnarf, 1929), the Asphodeloideae (Schnarf and Wunderlich, 1939; Cave, 1953), the Scilloideae (Wunderlich, 1937; Buchner, 1948), and the Aletroideae (Schnarf, 1929), as well as in some genera of the family Amaryllidaceae (Schnarf, 1931; Stenar, 1951), to which the Allioideae is referred by Hutchinson (1959).

From a taxonomic point of view, the difference in embryological characters that exists between *Muilla* and other genera of the Allieae, particularly *Allium,* is important since it indicates that *Muilla* certainly is more distantly related to *Allium* and the other Allieae here discussed than is now generally assumed. For lack of information about the embryological variation within the Allieae as a whole, this line of thought cannot possibly be pursued any further at this time. For the same reason a detailed elaboration on the embryologic similarity and possible relationship of *Muilla* to one or more genera of the Melanthioideae, Asphodeloideae, Scilloideae, Aletroideae, or Amaryllidaceae *sensu* Krause will have to wait.

Summary

The ovule is anatropous, bitegmic, and crassinucellate. The inner integument consists of two, the outer of four to five, layers of cells. The micropyle is formed by the inner integument only, but after fertilization the outer integument elongates considerably and forms a characteristic protrusion toward the placenta. Periclinal walls occur in the nucellar epidermis, but the bulk of the nucellus is produced from the original subepidermal cells. At the time of fertilization, the massive nucellus is one-and-a-half times as long as the embryo sac and completely surrounds it. A parietal tissue of several cells is present. Embryo sac development is according to the *Polygonum* type. The endosperm is helobial.

Numerous differences in embryologic characters exist between *Muilla* and the other genera of the Allieae that have been investigated in this respect. Probably *Muilla* is more distantly related to these members of the Allieae than is realized at present. Embryologically, *Muilla* shows similarity with several genera of the subfamilies Melanthioideae, Asphodeloideae, Scilloideae, and Aletroideae of the Liliaceae, and with genera of the family Amaryllidaceae *sensu* Krause.

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